

INHIBITORS ARE NOT RESPONSIBLE FOR POOR GERMINATION OF *PROTEA COMPACTA* SEED

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ABSTRACT

Evidence is provided which shows that in the case of *Protea compacta* poor germination is not due to the presence of inhibitors but rather to a lack of promoters. It would appear as if butanol-soluble cytokinins and gibberellin-like substances together control the germinability of the seed.

UITTREKSEL

INHIBEERDERS IS NIE VERANTWOORDELIK VIR DIE SWAK ONTKIEMING VAN *PROTEA COMPACTA* SAAD NIE

Bewyse is gevind wat daarop dui dat in die geval van *Protea compacta* swak ontkieming nie te wyte is aan die teenwoordigheid van inhibeerders nie, maar eerder toegeskryf kan word aan 'n tekort aan ontkiemingstimuleerders. Dit wil voorkom asof butanol-oplosbare sitokiniene en gibberellienagtige verbindings gesamentlik die ontkiembaarheid van die saad beheer.

INTRODUCTION

Vogts in 1960 inferred that the poor germination of proteaceous seed could be explained by the presence of inhibitors. This assumption had an important influence on subsequent research in that it stimulated interest in the isolation of inhibitors but, perhaps unfortunately, it also directed attention away from other factors that could be responsible for reduced germinability, especially as dormancy can also be caused by a lack of promoters (Villiers and Wareing, 1965). Substances that inhibit lettuce seed germination can be leached and extracted from the seed of a number of Proteaceae species. The removal of these compounds however, did not result in any significant improvement of germination (Brown and Van Staden, 1971; Van Staden and Brown, 1972; 1973). As their removal must be correlated with an enhancement in germination in order for them to be considered as an obstacle to germination the question immediately arises whether these compounds are indeed involved in germination. To determine whether inhibitors have an overriding effect in regulating germination in *Protea compacta* seed, or whether germination is determined by a balance between inhibitors and promoters, the effects of favourable environmental factors on germination (Brown and Van Staden, 1973a) and on the levels of endogenously occurring growth substances were investigated.

MATERIALS AND METHODS

The seed of *Protea compacta* R. Br. used in the present investigation was purchased from the Department of Forestry, Pretoria.

Stratification and oxygen incubation

Seed was imbibed in distilled water for 24 hours. Samples were subsequently incubated on moist filter paper at 5°C and 25°C for 0, 30, 60 and 90 days respectively and then extracted for growth regulators. A second batch of seeds was placed on moist filter paper in 500 ml flasks. The flasks were evacuated and flushed daily with air, oxygen or nitrogen. Seeds were incubated in each gas for 0, 10, 15 and 20 days respectively in a germinator with temperatures alternating between 10°C for 8 hours and 20°C for 16 hours and subsequently extracted for growth regulators. Each experiment was repeated at least twice.

Germination data were obtained from separate samples of seed treated as described above.

Extraction and bioassay of growth regulators

Ten grammes of non-germinated embryo material were extracted for each hormone. Cytokinins were extracted and assayed by means of the soybean bioassay as previously described (Van Staden, Webb and Wareing, 1972).

Gibberellin-like substances were extracted using a technique based on that of Phillips (1972), but omitting the agar-diffusion step. Embryo material was extracted directly in 80% methanol. The aqueous extracts obtained were partitioned with three equal volumes of redistilled ethyl acetate at pH 2.5. The pooled ethyl acetate fraction, containing acidic and possibly some of the neutral gibberellins, was reduced to dryness under vacuum at 35°C and the residue taken up in a small volume of ethyl acetate.

For inhibitors, embryo material was extracted as for gibberellins, except that the acidified aqueous extract was partitioned with redistilled ether.

Both the gibberellin and inhibitor extracts were strip-loaded onto Whatman No. 1 chromatography paper and separated with *iso*-propanol: ammonia: water (10:1:1 v/v). The dried chromatograms were divided into 10 equal strips. Gibberellin-like activity in the acidic ethyl acetate fractions was determined in the *Rumex* leaf senescence assay developed by Whyte and Luckwill (1966). Inhibitor levels were determined using the lettuce seed germination assay (Sankhla and Sankhla, 1968).

RESULTS

Figure 1 shows that incubation of seed in oxygen or at a low temperature enhanced germination of *Protea compacta* seed. Although there were minor fluctuations in the levels of inhibitors in the acidic ether and aqueous fractions of embryo extracts, no major trends of changes in inhibitor levels after incuba-

tion at 5°C or 25°C were recorded (Fig. 2). The results obtained with incubation in air, oxygen and nitrogen showed similar trends and are therefore not presented. However, incubation at low temperatures (Fig. 3) and in the presence of various gases (Fig. 4) resulted in increases in promoter levels. In the case of the former treatment the level of butanol-soluble cytokinins reached a peak after 30 days of chilling and the level of acidic gibberellin-like substances increased progressively over a period of 60 days. The highest level of butanol-soluble cytokinins was correlated with maximum germination which was 50% higher than that of the control (Fig. 1B). Incubation at 25°C resulted in a drop in the levels of both promoters. The fact that this decrease in promoter levels was correlated with a very low germination percentage suggests that poor germination may be associated with a lack of promoters rather than the presence of inhibitors.

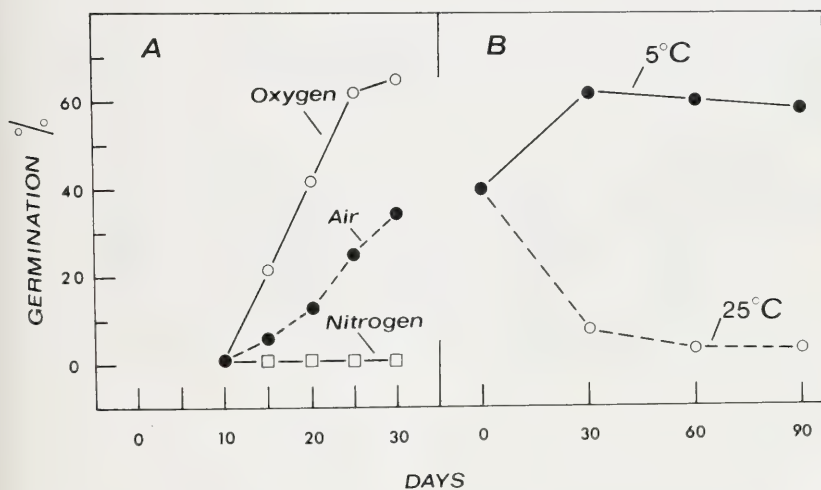


FIG. 1.

A. The effect of air, oxygen and nitrogen on the germination of *Protea compacta* seed. B. Germination of seed of *Protea compacta* after incubation at 5°C and 25°C for 0, 30, 60 and 90 days, respectively.

The levels of butanol-soluble cytokinins dropped during the first ten days of incubation in air, oxygen and nitrogen (Fig. 4). Thereafter the levels increased again relative to the ten-day levels, each reaching a peak at 15 days. However, whereas the peak level in oxygen represented an increase of 16% over the level of the control, the peaks in air and nitrogen were 20% and 40% below the control,

respectively. The increase in butanol-soluble cytokinins with oxygen treatment occurred just before a period in which the maximum rate of germination occurred (Fig. 1A). Sixty-seven percent of the seeds germinated in oxygen, whereas 40% germinated in air and no germination took place in nitrogen.

Incubation in the different gases resulted in a similar trend in the levels of acidic gibberellin-like substances. A peak of activity was shown in each gas at 15 days (Fig. 4). However, this increase in gibberellin-like substances appeared to improve germination only when accompanied by a simultaneous increase in butanol-soluble cytokinins (oxygen incubation).

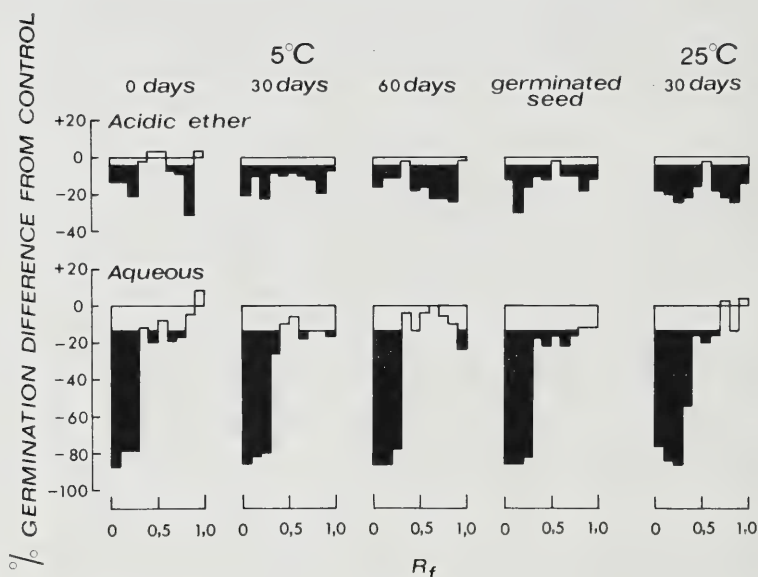


FIG. 2.

Inhibitors from acidic ether and aqueous fractions of embryo extracts of *Protea compacta* seed incubated at 5°C and 25°C, respectively. Extracts were chromatographed on paper in *iso*-propanol: ammonia: water (10:1:1v/v) and assayed with the Grand Rapids lettuce seed germination bioassay. Shaded areas represent differences significant from water controls at the 1% level.

DISCUSSION

The levels of inhibitors extracted from embryos were similar in treatments which gave maximum germination and those in which no germination occurred. It thus seems reasonable to assume that the inhibitors extracted were not of

importance in the regulation of germination. These results support the findings of leaching experiments (Brown and Van Staden, 1973b) and the report that poor germination of seed of *Leucospermum cordifolium* cannot be directly attributed to the presence of inhibitors (Van Staden and Brown, 1973).

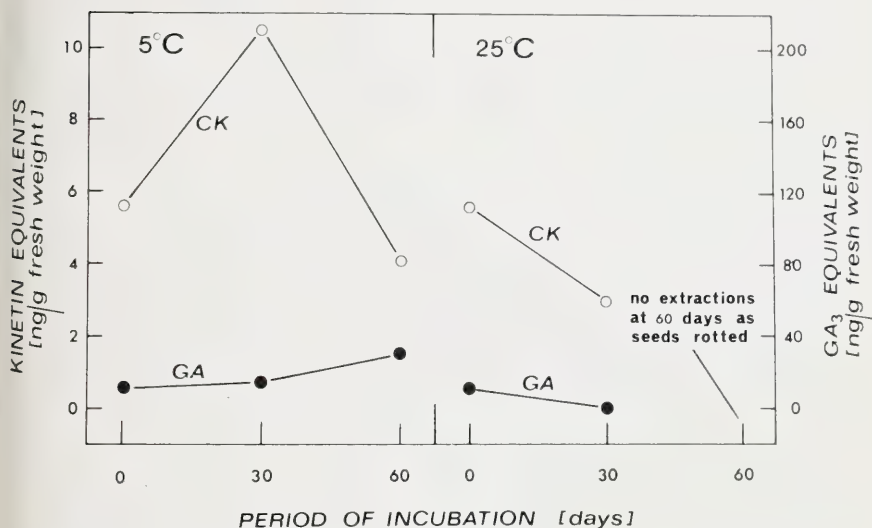


FIG. 3.

The effect of incubation at 5°C and 25°C on the levels of butanol-soluble cytokinins and acidic gibberellin-like substances in seed of *Protea compacta*. Butanol-soluble cytokinins measured by the soybean callus bioassay (CK). Acidic gibberellin-like substances measured by the *Rumex* leaf senescence retardation bioassay (GA).

Increases in promoter levels were correlated with maximum germination suggesting that poor germinability in this species is mainly due to a lack of promoters. Both butanol-soluble cytokinins and acidic gibberellin-like substances are apparently required for germination. This differs from the hypothesis of Khan (1971) in which gibberellin was regarded as the primary stimulus needed for germination. In seed of *Protea compacta* gibberellins do not appear to be primarily responsible for improved germination. In the absence of oxygen, a condition under which no germination occurred, cytokinin levels were low, suggesting that the synthesis of these compounds is oxygen dependent.

ACKNOWLEDGMENTS

The financial assistance of the C.S.I.R. Pretoria is gratefully acknowledged.

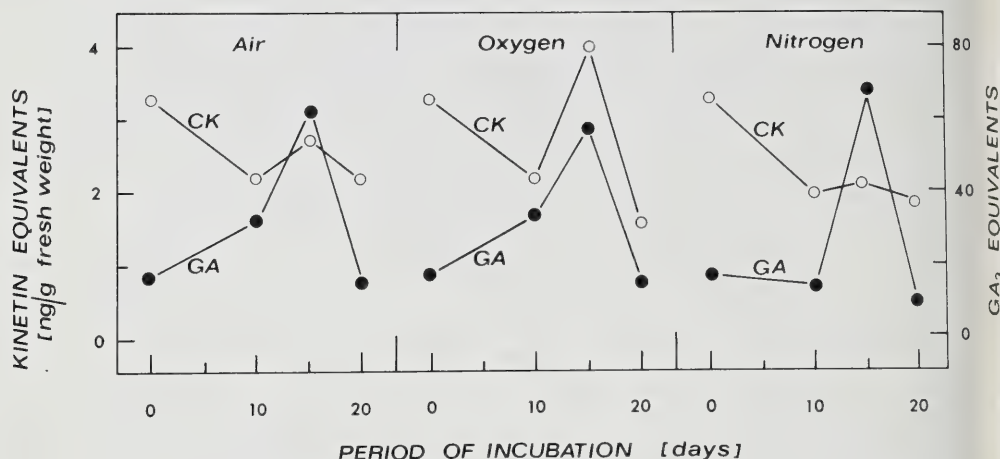


FIG. 4.

The effect of incubation in air, oxygen and nitrogen on the level of butanol-soluble cytokinins and acidic gibberellin-like substances in seed of *Protea compacta*. Butanol-soluble cytokinins measured by the soybean callus bioassay (CK). Acidic gibberellin-like substances measured by the *Rumex* leaf senescence retardation bioassay (GA).

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